

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	84	ketoisophorone	US-PGPUB; USPAT; DERWENT	OR	OFF	2005/12/02 14:47
L2	3	ketoisophorone and dehydrogenase	US-PGPUB; USPAT; DERWENT	OR	OFF	2005/12/02 14:49
L3	22	old adj yellow adj enzyme	US-PGPUB; USPAT; DERWENT	OR	OFF	2005/12/02 14:49
L4	9	I3 and cerevisiae	US-PGPUB; USPAT; DERWENT	OR	OFF	2005/12/02 14:50

L8 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN  
 AN 2003:678999 CAPLUS  
 DN 139:213015  
 TI Enzymatic process for producing levodione from ketoisophorone  
 IN Shimizu, Sakayu; Wada, Masaru  
 PA Roche Vitamins A.-G., Switz.  
 SO PCT Int. Appl., 18 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003070959	A2	20030828	WO 2003-EP1537	20030215
	WO 2003070959	A3	20031016		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	CA 2474802	AA	20030828	CA 2003-2474802	20030215
	EP 1476559	A2	20041117	EP 2003-742456	20030215
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK			
	JP 2005517448	T2	20050616	JP 2003-569851	20030215
	US 2005244941	A1	20051103	US 2005-505314	20050411
PRAI	EP 2002-3968	A	20020222		
	WO 2003-EP1537	W	20030215		
OS	CASREACT 139:213015				

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L6 ANSWER 1 OF 7 MEDLINE on STN  
 ACCESSION NUMBER: 2004496703 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15464593  
 TITLE: Cloning and overexpression of the old yellow enzyme gene of Candida macedoniensis, and its application to the production of a chiral compound.  
 AUTHOR: Kataoka Michihiko; Kotaka Atsushi; Thiwthong Rungruedee; Wada Masaru; Nakamori Shigeru; Shimizu Sakayu  
 CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan.. kataoka@kais.kyoto-u.ac.jp  
 SOURCE: Journal of biotechnology, (2004 Oct 19) 114 (1-2) 1-9. Journal code: 8411927. ISSN: 0168-1656.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200503  
 ENTRY DATE: Entered STN: 20041007  
 Last Updated on STN: 20050309  
 Entered Medline: 20050308  
 AB The gene encoding old yellow enzyme (OYE), which catalyzes the conversion of ketoisophorone (KIP; 2,6,6-trimethyl-2-cyclohexen-1,4-dione) to (6R)-levodione (2,2,6-trimethylcyclohexane-1,4-dione), of Candida

macedoniensis was cloned and sequenced. A 1212bp nucleotide fragment (oye) was confirmed to be the gene encoding OYE based on the agreement of internal amino acid sequences. Oye encodes a total 403 amino acid residues, and the deduced amino acid sequence shows a high degree of similarity to those of other microbial OYE family proteins. An expression vector, pETOYE, that contains the full length of oye was constructed. Escherichia coli harboring pETOYE exhibited an about six-fold increase in specific KIP-reducing activity under the control of the T7 promoter as compared with that of C. macedoniensis. (6R)-Levodione formed with washed cells of the transformant and a cofactor regeneration system amounted to 638 mM (98.2 mg ml<sup>-1</sup>), the a molar yield being 96.9%. The asymmetric reduction of KIP to (6R)-levodione with E. coli cells, which co-expressed both oye and the glucose dehydrogenase gene (gdh), as a catalyst was investigated. The (6R)-levodione formed amounted to 627 mM (96.6 mg ml<sup>-1</sup>), the a molar yield being 95.4%. Since the use of E. coli BL21 (DE3) cells co-expressing oye and gdh as a catalyst is simple and does not require the addition of glucose dehydrogenase, it is highly advantageous for the practical synthesis of (6R)-levodione.

L6 ANSWER 2 OF 7 MEDLINE on STN  
 ACCESSION NUMBER: 2003085163 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12596862  
 TITLE: Old Yellow Enzyme from Candida macedoniensis catalyzes the stereospecific reduction of the C=C bond of ketoisophorone.  
 AUTHOR: Kataoka Michihiko; Kotaka Atsushi; Hasegawa Akiko; Wada Masaru; Yoshizumi Ayumi; Nakamori Shigeru; Shimizu Sakayu  
 CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.. kataoka@kais.kyoto-u.ac.jp  
 SOURCE: Bioscience, biotechnology, and biochemistry, (2002 Dec) 66 (12) 2651-7.  
 Journal code: 9205717. ISSN: 0916-8451.  
 PUB. COUNTRY: Japan  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200308  
 ENTRY DATE: Entered STN: 20030225  
 Last Updated on STN: 20030813  
 Entered Medline: 20030812

AB Microorganisms were screened for ones that reduced 3,5,5-trimethyl-2-cyclohexene-1,4-dione (ketoisophorone; KIP), and several strains were found to produce (6R)-2,2,6-trimethylcyclohexane-1,4-dione (levodione). The enzyme catalyzing the reduction of the C=C bond of KIP to yield (6R)-levodione was isolated from Candida macedoniensis AKU4588. The results of primary structural analysis and its enzymatic properties suggested that the enzyme might be an Old Yellow Enzyme family protein.

L6 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 ACCESSION NUMBER: 2005:46260 BIOSIS  
 DOCUMENT NUMBER: PREV200500045568  
 TITLE: Cloning and overexpression of the old yellow enzyme gene of Candida macedoniensis, and its application to the production of a chiral compound.  
 AUTHOR(S): Kataoka, Michihiko [Reprint Author]; Kotaka, Atsushi; Thiwthong, Rungruedee; Wada, Masaru; Nakamori, Shigeru; Shimizu, Sakayu  
 CORPORATE SOURCE: Div Appl Life SciGrad Sch AgrSakyo Ku, Kyoto Univ, Kyoto, 6068502, Japan kataoka@kais.kyoto-u.ac.jp  
 SOURCE: Journal of Biotechnology, (October 19 2004) Vol. 114, No. 1-2, pp. 1-9. print.  
 ISSN: 0168-1656 (ISSN print).

DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 26 Jan 2005  
Last Updated on STN: 26 Jan 2005

AB The gene encoding old yellow enzyme (OYE), which catalyzes the conversion of ketoisophorone (KIP; 2,6,6-trimethyl-2-cyclohexen-1,4-dione) to (6R)-levodione (2,2,6-trimethylcyclohexane-1,4-dione), of *Candida macedoniensis* was cloned and sequenced. A 1212 bp nucleotide fragment (oye) was confirmed to be the gene encoding OYE based on the agreement of internal amino acid sequences. Oye encodes a total 403 amino acid residues, and the deduced amino acid sequence shows a high degree of similarity to those of other microbial OYE family proteins. An expression vector, pETOYE, that contains the full length of oye was constructed. *Escherichia coli* harboring pETOYE exhibited an about six-fold increase in specific KIP-reducing activity under the control of the T7 promoter as compared with that of *C. macedoniensis*. (6R)-Levodione formed with washed cells of the transformant and a cofactor regeneration system amounted to 638 mM (98.2 mg ml<sup>-1</sup>), the a molar yield being 96.9%. The asymmetric reduction of KIP to (6R)-levodione with *E. coli* cells, which co-expressed both oye and the glucose dehydrogenase gene (gdh), as a catalyst was investigated. The (6R)-levodione formed amounted to 627 mM (96.6 mg ml<sup>-1</sup>), the a molar yield being 95.4%. Since the use of *E. coli* BL21 (DE3) cells co-expressing oye and gdh as a catalyst is simple and does not require the addition of glucose dehydrogenase, it is highly advantageous for the practical synthesis of (6R)-levodione. Copyright 2004 Elsevier B.V. All rights reserved.

L6 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:819510 CAPLUS  
DOCUMENT NUMBER: 142:92286  
TITLE: Cloning and overexpression of the old yellow enzyme gene of *Candida macedoniensis*, and its application to the production of a chiral compound  
AUTHOR(S): Kataoka, Michihiko; Kotaka, Atsushi; Thiwthong, Rungruedee; Wada, Masaru; Nakamori, Shigeru; Shimizu, Sakayu  
CORPORATE SOURCE: Graduate School of Agriculture, Division of Applied Life Sciences, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan  
SOURCE: Journal of Biotechnology (2004), 114(1-2), 1-9  
CODEN: JBITD4; ISSN: 0168-1656  
PUBLISHER: Elsevier B.V.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The gene encoding old yellow enzyme (OYE), which catalyzes the conversion of ketoisophorone (KIP; 2,6,6-trimethyl-2-cyclohexen-1,4-dione) to (6R)-levodione (2,2,6-trimethylcyclohexane-1,4-dione), of *Candida macedoniensis* was cloned and sequenced. A 1212 bp nucleotide fragment (oye) was confirmed to be the gene encoding OYE based on the agreement of internal amino acid sequences. Oye encodes a total 403 amino acid residues, and the deduced amino acid sequence shows a high degree of similarity to those of other microbial OYE family proteins. An expression vector, pETOYE, that contains the full length of oye was constructed. *Escherichia coli* harboring pETOYE exhibited an about six-fold increase in specific KIP-reducing activity under the control of the T7 promoter as compared with that of *C. macedoniensis*. (6R)-Levodione formed with washed cells of the transformant and a cofactor regeneration system amounted to 638 mM (98.2 mg ml<sup>-1</sup>), the a molar yield being 96.9%. The asym. reduction of KIP to (6R)-levodione with *E. coli* cells, which co-expressed both oye and the glucose dehydrogenase gene (gdh), as a catalyst was investigated. The (6R)-levodione formed amounted to 627 mM (96.6 mg ml<sup>-1</sup>), the a molar yield being 95.4%. Since the use of *E. coli* BL21 (DE3) cells co-expressing oye and gdh as a catalyst is simple and does not require the addition of glucose dehydrogenase, it is highly

advantageous for the practical synthesis of (6R)-levodione.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:678999 CAPLUS

DOCUMENT NUMBER: 139:213015

TITLE: Enzymatic process for producing levodione from ketoisophorone

INVENTOR(S): Shimizu, Sakayu; Wada, Masaru

PATENT ASSIGNEE(S): Roche Vitamins A.-G., Switz.

SOURCE: PCT Int. Appl., 18 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003070959	A2	20030828	WO 2003-EP1537	20030215
WO 2003070959	A3	20031016		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
CA 2474802	AA	20030828	CA 2003-2474802	20030215
EP 1476559	A2	20041117	EP 2003-742456	20030215
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK			
JP 2005517448	T2	20050616	JP 2003-569851	20030215
US 2005244941	A1	20051103	US 2005-505314	20050411
PRIORITY APPLN. INFO.:			EP 2002-3968	A 20020222
			WO 2003-EP1537	W 20030215

OTHER SOURCE(S): CASREACT 139:213015

AB An enone reductase characterized by a mol. mass of 61,300 ± 5,000 Da; NADPH and NADH as co-factor; a temperature optimum of 55-60°C at pH 7.4; a pH optimum of 4.5-8.5 and a substrate specificity on α,β-unsatd. ketones, especially derived from a yeast and a process for the preparation of levodione from ketoisophorone. Thus, NADH dehydrogenase from *Saccharomyces cerevisiae* were identified from genomic DNA using primers for the genes *oye2* and *oye3*. The *oye2* or the *oye3* gene was then cloned into *Escherichia coli* JM109 using the pKK223-3 plasmid. Cells were grown, induced with IPTG and harvested by centrifugation. The harvested cells were lysed by sonication and the supernatant recovered. This cell free extract was then used to reduce ketoisophorone to levodione.

L6 ANSWER 6 OF 7 LIFESCI COPYRIGHT 2005 CSA on STN

ACCESSION NUMBER: 2005:15234 LIFESCI

TITLE: Cloning and overexpression of the old yellow enzyme gene of *Candida macedoniensis*, and its application to the production of a chiral compound

AUTHOR: Kataoka, M.; Kotaka, A.; Thiwthong, R.; Wada, M.; Nakamori, S.; Shimizu, S.

CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan; E-mail:

kataoka@kais.kyoto-u.ac.jp  
SOURCE: Journal of Biotechnology [J. Biotechnol.], (20041000) vol. 114, no. 1-2, pp. 1-9.  
ISSN: 0168-1656.  
DOCUMENT TYPE: Journal  
FILE SEGMENT: W2; K  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The gene encoding old yellow enzyme (OYE), which catalyzes the conversion of **ketoisophorone** (KIP; 2, 6, 6-trimethyl-2-cyclohexen-1, 4-dione) to (6R)-levodione (2, 2, 6-trimethylcyclohexane-1, 4-dione), of *Candida macedoniensis* was cloned and sequenced. A 1212 bp nucleotide fragment (oye) was confirmed to be the gene encoding OYE based on the agreement of internal amino acid sequences. Oye encodes a total 403 amino acid residues, and the deduced amino acid sequence shows a high degree of similarity to those of other microbial OYE family proteins. An expression vector, pETOYE, that contains the full length of oye was constructed. *Escherichia coli* harboring pETOYE exhibited an about six-fold increase in specific KIP-reducing activity under the control of the T7 promoter as compared with that of *C. macedoniensis*. (6R)-Levodione formed with washed cells of the transformant and a cofactor regeneration system amounted to 638 mM (98.2 mg ml super(-1)), the a molar yield being 96.9%. The asymmetric reduction of KIP to (6R)-levodione with *E. coli* cells, which co-expressed both oye and the glucose dehydrogenase gene (gdh), as a catalyst was investigated. The (6R)-levodione formed amounted to 627 mM (96.6 mg ml super(-1)), the a molar yield being 95.4%. Since the use of *E. coli* BL21 (DE3) cells co-expressing oye and gdh as a catalyst is simple and does not require the addition of glucose dehydrogenase, it is highly advantageous for the practical synthesis of (6R)-levodione.

L6 ANSWER 7 OF 7 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2004418287 EMBASE  
TITLE: Cloning and overexpression of the old yellow enzyme gene of *Candida macedoniensis*, and its application to the production of a chiral compound.  
AUTHOR: Kataoka M.; Kotaka A.; Thiwthong R.; Wada M.; Nakamori S.; Shimizu S.  
CORPORATE SOURCE: M. Kataoka, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto Univ., Kitashirakawa-O., Kyoto, Japan. kataoka@kais.kyoto-u.ac.jp  
SOURCE: Journal of Biotechnology, (19 Oct 2004) Vol. 114, No. 1-2, pp. 1-9.  
Refs: 28  
ISSN: 0168-1656 CODEN: JBITD4  
PUBLISHER IDENT.: S 0168-1656(04)00251-2  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20041018  
Last Updated on STN: 20041018

AB The gene encoding old yellow enzyme (OYE), which catalyzes the conversion of **ketoisophorone** (KIP; 2,6,6-trimethyl-2-cyclohexen-1,4-dione) to (6R)-levodione (2,2,6-trimethylcyclohexane-1,4-dione), of *Candida macedoniensis* was cloned and sequenced. A 1212 bp nucleotide fragment (oye) was confirmed to be the gene encoding OYE based on the agreement of internal amino acid sequences. Oye encodes a total 403 amino acid residues, and the deduced amino acid sequence shows a high degree of similarity to those of other microbial OYE family proteins. An expression vector, pETOYE, that contains the full length of oye was constructed. *Escherichia coli* harboring pETOYE exhibited an about six-fold increase in specific KIP-reducing activity under the control of the T7 promoter as

compared with that of *C. macedoniensis*. (6R)-Levodione formed with washed cells of the transformant and a cofactor regeneration system amounted to 638 mM (98.2 mg ml<sup>-1</sup>), the a molar yield being 96.9%. The asymmetric reduction of KIP to (6R)-levodione with *E. coli* cells, which co-expressed both *oye* and the glucose dehydrogenase gene (*gdh*), as a catalyst was investigated. The (6R)-levodione formed amounted to 627 mM (96.6 mg ml<sup>-1</sup>), the a molar yield being 95.4%. Since the use of *E. coli* BL21 (DE3) cells co-expressing *oye* and *gdh* as a catalyst is simple and does not require the addition of glucose dehydrogenase, it is highly advantageous for the practical synthesis of (6R)-levodione. .COPYRGHT. 2004 Elsevier B.V. All rights reserved.